Electrical wave transmission in healthy black pine seedlings

GUO Jian[1,2,3], ZHAO Bo-guang[3], ZHOU Kai-ya[2]

[1] *Haikou Bureau of Forestry, Hainan, P. R. China*

[2] *College of Life Science, Nanjing Normal University, Nanjing 210049, P. R. China*

[3] *Forest Resource & Environment College of Nanjing Forestry University, Nanjing 210037, P. R. China*

Abstract: Non-injurious local stimuli, such as a cold shock, and injurious stimuli, such as local burning, punctures or chemicals, were applied to study electrical wave transmission in black pine (*Pinus thunbergii*) seedlings. The results showed that non-injurious stimuli evoked the action potential (AP) transmission and injurious stimulation induced both AP transmission and the more complex variation potential (VP) transmission in the seedlings. The causes of these phenomena were discussed.

Key words: Black pine; *Pinus thunbergii*; Action potential (AP); Variation potential (VP); Electrical wave transmission

CLC number: S791.256 **Document code**: A **Article ID**: 1007-662X(2004)02-0135-03

Introduction

Ever since electrical activity in plants was examined by Burdon-Sanderson in 1873 (Burdon 1873), increasing attention has been given to plant electrophysiology. The phenomenon of electrical wave (EW) transmission has been found in many kinds of plants, including fungi (Slayman *et al.* 1976), algae (Hope *et al.* 1975; Gradmann *et al.* 1980), and higher plants (Van Sambeek *et al.* 1976; Roblin 1979, 1985a, b; Simons 1981; Pickard 1973; Zawadzki 1980; Davies *et al.* 1981). Electrical waves can be induced by light (Stolarek *et al.* 1980; Paszewski 1976), heat (Sakamoto *et al.* 1984), cold (Pickard 1973), chemicals (Kishimoto 1966), mechanical stimuli (Paszewski *et al.* 1973), electrical stimuli (Pickard 1971), and wounding (Roblin 1985a, b). These observations lead to the hypothesis that electrical wave transmission is universal in plants and perhaps is the first response of plants to outside stimuli. This response can affect many physiological processes of a plant, such as growth, respiration, and metabolism (Simons 1981; Pickard 1973; Lou 1955, 1960; Davies 1987), and thereby regulate the relationship between the plants and environment.

Most of these studies used sensitive plants, herbaceous plants or liane as test material. However, it is difficult to observe EW transmission in trees owing to their thick bark, thus reports involving trees are seldom found (Sakamoto 1984; Guo 1997, Formm 1993). We used tree seedlings in our experiments to overcome this problem. Healthy black pine (*Pinus thunbergii*) seedlings were used to study the electrical waveforms in response to non-injurious and injurious stimuli. Our objective was to compare waveforms in the hope of finding a new, electrophysiological method for early detection of disease in plants. A subsequent study will involve pine seedlings inoculated with pine wood nematode to further investigate this possibility.

Materials and methods

Plant material

Paper filter discs were placed in Petri dishes and soaked with water. Seeds of black pine were placed on the discs, and the Petri dishes incubated in a phytotron. The phytotron was maintained at 25°C, 65% relative humidity, and natural illumination. The experiment began after 10-15 days when new leaves first developed in the seedlings. Seedlings were then kept in the laboratory for one day (24 h) before using in experiments.

Electrical measurements

Non-polarizable calomel electrodes were used for extracellular measurements. The electrodes were placed in a conductive solution (Composition in mmol·L^{-1}: $MgCl_2$ 0.1, KCl 0.1, $CaCl_2$ 0.5, Na_2SO_4 0.05). The measuring electrode was connected to the exterior surface of the foliage of the pine tree and the reference electrode was connected to the surface of the stem. Contact between the electrodes and the plant was made through a segment of fine cotton thread wetted with saturated KCl solution (Fig. 1). The electrodes were brought into contact with the plant and the electrical signals were recorded with a physiological collection system (China Agriculture University, SLY-4). The electrical outputs were recorded and displayed on a computer.

During electrical measurements, the pine seedlings were moved to a growth cabinet kept at a constant temperature of 25 and 65% humidity, and with natural illumination. The seedling and recording apparatus were enclosed by Faraday cage to exclude extraneous electrical noise.

Foundation item: This paper was supported by the National Nature Science Foundation of China (grant No. 39670613).

Biography: GUO Jian (1971-), male, lecturer in Haikou Bureau of Forestry, Hainan, P. R. China. Email: jguo58@yahoo.com

Received date: 2003-10-16

Responsible editor: Song Funan

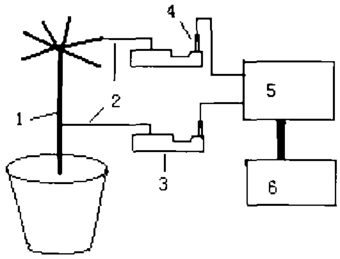

Fig. 1 Schematic diagram of the apparatus for extracellular measurement of black pine seedling
(1: Black pine seedling; 2: Cotton thread; 3: Electrode trough; 4: Electrode; 5: Recording system; 6: Computer)

Stimulation

In most cases, electrical waves can be induced in plants by various physical stimuli. In our experiment, we used cold shock as a non-injurious local stimulus, and a local burn, puncture, and chemicals as injurious stimuli to evoke electrical wave transmission in seedlings. For cold shock, we took out a piece of ice (0.5 cm×1.0 cm×2.0 cm) from a refrigerator (-17°C) and touched one part of the leaves for 1.5 s. To cause a local burn, the tip of a leaflet was passed over a flaming match for 1.5 s. A puncture was produced by inserting and removing a vitreous needle into the stem of the seedling. Chemical stimuli were obtained by touching the tip of a leaflet with either saturated NaOH or 98% H_2SO_4 for 1.5 s, respectively. Eight to ten replicates per stimulus were measured. Each replicate used a different seedling.

Results

EW transmission elicited by non-injurious stimulus

We observed typical action potential transmission in the black pine seedlings when we touched the leaflet with ice (Fig. 2). Usually waveforms ran above the baseline and but occasionally they ran below the baseline. These changes in the wave polarity appeared to be spontaneous – no cause was evident.

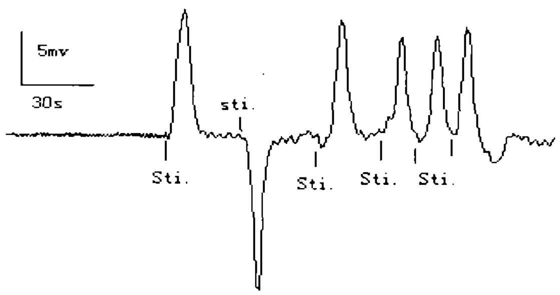

Fig. 2 Action potentials elicited by non-injurious stimulus-cold shock. (Sti = application of stimulus)

EW transmission elicited by injurious stimuli

EW transmission elicited by local burn or puncturing

The EW transmission could be observed in black seedlings when a localized burn was applied to the tip a leaflet or punctured by glass needle. Four kinds of major waveforms were obtained (Fig. 3).

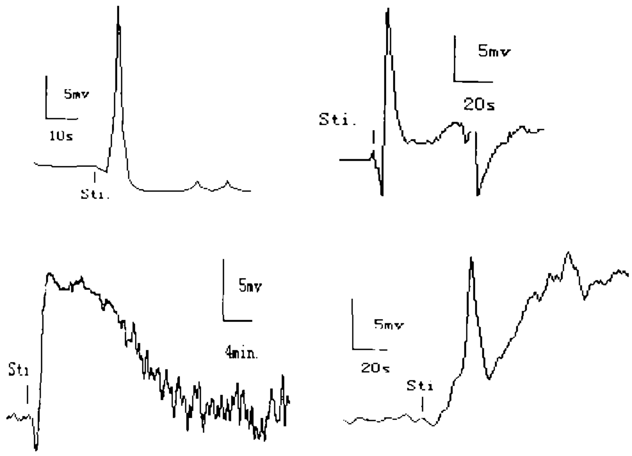

Fig. 3 The major wave forms elicited by local burn or puncturing

EW transmission elicited by strong acid and alkali

EW transmission could be observed when the leaflet was touched with saturated NaOH for 1.5 s or similarly with 98% H_2SO_4 solutions (Fig. 4).

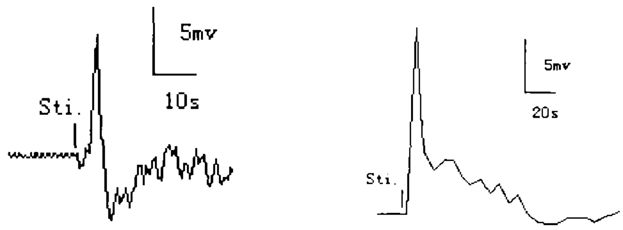

Fig. 4 EW transmission elicited by saturated NaOH or 98% H_2SO_4 solutions

Discussion

In general, two kinds of electrical responses to stimulation were distinguished: the action potential (AP) transmission in response to the non-injurious local stimulus (cold shock), and the variation potential (VP) transmission in response to the injurious stimuli (needle puncture, local burn, and chemicals). Differences between the AP and VP were observed in their waveform and rate and range of transmission. The transmission of AP had a higher velocity, which was often within a limited region and was in the form of a sharp spike. In contrast, the VP occurred more slowly over a much longer distance, and in the form of irregular spikes superimposed on a plateau, the magnitude and duration of which varied with stimulus intensity. The VP returned to the baseline gradually (Gradmann *et al.* 1980).

The AP requires living tissue for conduction (Pickard 1973), and travels through the xylem and phloem parenchyma by successive relays of the constituent cells interconnected by plasmodesmata. AP is not always transmitted throughout the entire plant and commonly stop at a node (Van Sambeek 1976). The VP has been associated with a chemical substance, Ricca's Factor (Pickard 1973).

Ricca's Factor apparently allows the VP to be propagated through dead tissue, unlike an AP. Ricca's Factor is believed to be released from the site of wounding and transported by transpiration flow. It elicits the VP as it moves and comes in contact with adjoining tissue. Ricca's Factor has been obtained from many plant extracts (Van Sambeek 1976). Van Sambeek *et al.* (1976) suggested that Ricca's Factor was a new class of hormone, Riccanin. Whether or not Ricca's Factor isolated from different plants is the substance has not been investigated.

Our results showed that non-injurious stimulation could induce AP transmission and injurious stimulation could elicit both AP and VP transmission in black pine seedlings. The AP frequency is very high when we applied the injurious stimuli to the seedlings.

It is well known that the spread of electrical excitation in the stem has action potential character; that is, it follows the basic laws of excitability known in electrophysiology, e.g. the all-or-nothing law, and refractory periods. Stadnik and Bobersky (1976) found that cold shock failed to elicit the EW transmission when the first cold shock was applied 2.5 min after the first stimulus. However, the same response could be obtained when the cold shock was applied 30-20 min after the first. But, in our study, no refractory period was observed. The application of a second cold stimulus to black pine seedlings immediately following the first elicited the same response as observed with the first stimulus.

In 1837, Dutrochet observed that chilling the cell quickly could stop the flow of the cytoplasm abruptly for a few minutes. Cook (1929) quick chilled the cell from 20°C to 10°C, 7°C, 5°C, 4°C and 3°C, the flow of the cytoplasm was stopped 0, 18%, 23%, 65%, and 100%, respectively. Hill (1935) hypothesized that electrical signals were linked to the cessation of cytoplasmic flow. The EW transmission could be observed when we touched the leaflet with a piece of ice but the value of voltage was sometimes positive and sometimes negative; however, the conditions causing this reversal in polarity are not understood. The phenomenon may be related to changes in cytoplasmic flow but further study need to be done.

Acknowledgements

We wish to express our grateful appreciation to Dr. Gary Grant of Canada Forest Service, Sault Ste. Marie for helpful review.

References

Burdon Sanderson. 1873. Note on the electrical phenomena which accompany stimulation of the leaf of *Dionaea muscipula* [J]. Proc. Roy. Soc. London, **21**:495-496.

Cook, S.F 1929. The effects of sudden changes in temperature on the protoplasmic streaming [J]. J. General Physiology, **12**: 793-803.

Davies, E. 1987. Action potentials as multifunctional signals in plants: a unifying hypothesis to explain apparently disparate wound responses [J]. Plant Cell and Environment, **10**: 623-631.

Davies, E. and Schuster, A. 1981. Intercellular communication in plants: Evidence for a rapidly generated, bidirectionally transmitted wound signal [J]. Proceedings of the National Academy of Science, U.S.A., **78**:2442-2426.

Formm, J. and Spanswick, R. 1993. Characteristics of action potentials in willow (*salix viminalis*) [J]. J. Exp. Bot., **264**: 1119-1125.

Gradmann, D. and Mummert, H. 1980. Plant action potentials. Plant Membtane Transport: Current Conceptual Issues [M]. Elsevier/NorthHolland Biomedical Press, pp333-347.

Guo Jinyao, Hua Baoguang, Lou Chenhou. 1997. VP transmission in willow [J]. Scientia silvae sinicae, **23**: 1-7.

Hill, S.E. 1935. Stimulation by cold in Nitella [J]. J. General Physiology, **18**: 357-367.

Hope, A.B. and Walker, N.A. 1975. Action potentials in charophyte cells. The physiology of Giant Algal Cells [M]. Cambridge: Cambridge University Press, pp111-119.

Kishimoto, U. 1966. Repetitive action potentials in *Nitella internodes.* Plant and Cell Phyiol, **7**: 547-558.

Lou Chenhou. 1955. Response of the plants and its function between the biology and environment [J]. Science Bulletin, **12**: 26-33.

Lou Chenhou. 1960. Response and movement of plants [J]. Physiological Bulletin, **1**: 1-7.

Minorsky, P.V 1989. Temperature sensing by plants: a review and hypothesis. Plant, Cell and Environment, **12**: 119-135.

Paszewski, A. and Zawadzki, T. 1973. Action potentials in *Lupinus angustifolius* shoots [J]. J. Exp. Bot., **24**: 804-809.

Paszewski, A. and Zawadzki, T. 1976. Action potentials in *Lupinus angustifolius* shoots Determination of the refractory periods [J]. J. Exp. Bot., **27**: 369-374.

Pickard, B.G. 1971. Action potentials resulting from mechanical stimulation of pea epicotyls [J]. Planta, **97**: 106-115.

Pickard, B.G. 1973. Action potential in higher plants [J]. Bot. Rev. **39**: 172-201.

Roblin, G. 1979. Mimosa pudica: a model for the study of the excitability in plants [J]. Biol. Rev., **54**: 135-153.

Roblin, G. 1985a. Analysis of the variation potential induced by wounding in plants [J]. Plant Cell Physiol., **26**: 455-461.

Roblin, G. 1985b. Propogation in *Vicia faba* stem of a potential variation induced by wounding [J]. Plant Cell Phsiol, **26**: 1273-1283.

Sakamoto, M and Sumiya, K. 1984. The bioelectrical potentials fo young woody plants [J]. Wood Research, **70**: 42-46.

Simons, P.J. 1981. The role of electricity in plant movements [J]. New Phytologist, **87**: 11-37.

Slayman, C.L. Long, W.S. and Gradman, D. 1976. Action potentials in *Newrospara crassa* a mycelial fungus [J]. Biochimica et Biophysica Acta, **426**: 732-744.

Stadnik, S.A and Bobersky, G.A 1976. Bioelectric responses of plants to impulse temperature effects [J]. Biulletin Gosudartsvennyi Nikitskii Botanicheskii Sad., **29**: 43-48.

Stolarek, J. Jablonska, B and Pazurbiewicz Kocot. K. 1980. Light induced action potentials in Phaseolus Vulgaris. Plant membrane Transport: Current conceptual Issues [M]. Elsevier North Holl. Biomedical Press.

Van Sambeek, J.W. and Pickard, B.G. 1976. Mediation of rapid electrical metbolic transpirational and photosynthetic changes by factor released from wounds [J]. Canadian Journal of Botany, **54**: 2642-2671.

Wildon, D.C. Thain, J.F. and Minchin, P.E.H. 1992. Electrical signaling and systemic proteinase inhibitor induction in the wounded plant [J]. Nature, **360**: 62-65.

Zawadzki, T. 1980. Action potentials in Lupinus angustifolius. Spread of excitation in the stem. Leaves and root [J]. J. Exp. Bot, **31**: 1371-1377.